IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application No. 09/803,578

Applicant: Hwu et al.

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RESPONSE UNDER 37 CFR 1.116
EXPEDITED PROCEDURE

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DECLARATION UNDER 37 C.F.R. § 1.131 OF PATRICK K. HWU

I, Patrick K. Hwu, do hereby declare as follows:

1. I am a co-inventor of pending claims in the above-referenced patent application, along with Steven Rosenberg and Michael Kershaw.

2. We conceived of and reduced to practice the claimed invention in the United States before May 1, 2000, as evidenced by the following:

a. attached to this Declaration is a true and accurate copy of a report prepared by another co-inventor, Michael Kershaw, who worked in my laboratory. The report, which was prepared prior to May 1, 2000, summarizes research performed involving the production and testing of dual specificity T lymphocytes, which comprised the MOVγ chimeric receptor, specific for folate binding protein (FBP), and an endogenous T cell receptor, which is reactive with allogeneic cells.

3. The date deleted from the report is prior to May 1, 2000.

4. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 11/14/06

Patrick K. Hwu, M.D.

Generation of T cells with dual specificity.

Hwu lab meeting

The limited success of adoptive immunotherapy may be due to poor persisitence and/or loss of function of adoptively transferred cells in vivo. A remedy for this may be to generate T cells with dual specificity where one specificity can be used to target tumor and the other specificity enables expansion/activation in response to a powerful immunogen.

Dual specific mouse T cells:

Aim: to raise allo-specific (H-2b anti H-2d) T cells by MLR and adoptively transfer these in vitro grown cells and see if their persistence in vivo is increased by immunization with H-2d splenocytes.

Raising T cells: 2e6 irradiated BALB/c splenocytes + 2e6 C57BL/6 splenocytes (Thy 1.1) to each 24-well (4 plates). Fed IL-2 every 2 days and split as necessary. Restim on dat 8. Day 14 16 plates ready for adoptive transfer.

In vitro function (IFN-g secretion) in response to incubation with H-2d targets:

	media	MLR cells
media	0	0
B16	0	0
24JK	0	0
CT26	0	**7560**
C57BL/6 spleen	0	0
BALB/c spleen	0	**20230**

Phenotype:

In vivo trafficking/persistence: 1e7 Thy 1.1 CTL transferred iv into Thy 1.2 C57BL/6 mice in the presence or absence of immunization with approximately 5e7 BALB/c splenocytes either iv or sq (flank and footpads). Results are expressed as amount of Thy 1.1 cells in the tissue as a percentage of total cells in the that tissue:

Thy 1.1 CTL	Immunization	Spleen		Lung		Lymph node	
		Mean	SD	Mean	SD	Mean	SD
Yes	Triple iv	0.44	0.04	0.61	0.18		
Yes	single iv	0.40	0.12	0.22	0.04		
Yes	Triple sq	**4.94**	1.92	**1.06**	0.18	**11.62**	1.77
Yes	single sq	1.57	0.73	0.44	0.14	4.32	4.15
Yes	none	0.69	0.12	0.24	0.10		
No	triple iv	0.00		0.00			
No	single iv	0.00		0.00			
No	triple sq	0.00		0.00		0.00	
No	single sq	0.00		0.00		0.00	
No	none	0.00		0.00		0.00	
Yes	Triple iv	**33.06**	10.82	**10.66**	7.62		
Yes	none	10.92	2.83	2.62	1.46		
No	triple iv	0.00		0.00			

Conclude that:

1. sq immunization increases % Thy 1.1 in spleen

2. triple immunization is better
3. iv immunization ineffective for spleen
4. iv immunization augments Thy 1.1 in spleens of irradiated mice
5. triple immunization may have a slight effect on numbers in lung
6. iv immunization augments numbers in lungs of irradiated mice

Having established that immunization can increase numbers of in vitro generated allo-specific CTL the next step is to make these CTL dual specific for ovarian cancer with a chimeric receptor (MOv-gamma).

First attempt at transducing MLR failed as reported last time (coculture of MLR with GP + E86 MOvg packaging cells)

Therefore next tried a different method to get rapidly dividing mouse T cells for transduction using PHA and coculture for 5 days. Pheotype:

Restimed on day 12, FACS day 17:

Cells are CD8 but have lost MOvg expression

IFN-g release on day 18:

	media	SAMEN transduced	Movg transduced
Media	3	443	220
B16	1	162	66
CT26	0	808	3545
3T3	1	991	786
24JK	0	134	67
24JK-FBP	6	180	383
IGROV	0	218	1226
888 Mel	0	315	211

Cells have alloreactivity and some FBP reactivity.

Generation of dual (MOvg/Flu, MOvg/EBV) specific human T cells:

In this study M1 Flu specific or EBV-specific human T cells were generated and then transduced with chimeric MOvg. The hypothesis is that these T cells could be transferred to ovarian cancer patients and that these cells would expand/activate in response to a subsequent immunization with M1 Flu or EBV peptide.

Flu specific T cells were generated by incubation of PBMC with 1 micromolar M1. These were restimulated after 7 days with irradiated autologous M1 pulsed PBMC. 2 days later retroviral supernatant, IL-2, HEPBS, polybrene were added and spinoculated. Spinoculation was repeated the next day. G418 was added 2 days later. G418 removed after 6 days. Coculture performed 2 days later.

Last meeting described 20 MOv specific clones but Flu specificity was absent. All clones except 1 were CD4 therefore it is not surprising there was no M1 reactivity.

A new culture of MK PBMC was set up with M1 and transduced following a restim as done in the first culture (which gave great expression of MOv after G418 selection) but to ensure that CD4s did not overrun this culture CD8 were enriched 2 days after transduction. Very few Cd8 cells were got and these did not thrive.

Two further M1 cultures were started with new donors. One of these cultures was transduced on days 3 and 4 of this initial stim and then examined for MOv expression before G418 but it only had about 5% expression.

These two cultures were restimed after 8 days and transduced 2 and three days later with MOvg. They have been selected in G418 for 5 days and then grown for a further 3 days now so it is time to look for expression and reactivity. Initial FACS showed about 50:50 CD4 and CD8.

If dual reactivity of bulks is demonstrated then these cells need to be cloned and/or stained with flu tetramer.